ELSEVIER

Journal of Chromatography A, 871 (2000) 163–172

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Enantioselective analysis of methadone in saliva by liquid chromatography–mass spectrometry

Didier Ortelli[a], Serge Rudaz[a], Anne-Françoise Chevalley[b], Annie Mino[b], Jean-Jacques Deglon[c], Luc Balant[d], Jean-Luc Veuthey[a,*]

[a]Laboratory of Pharmaceutical Analytical Chemistry, University of Geneva, Boulevard d'Yvoy 20, 1211 Geneva 4, Switzerland
[b]Division d'abus de substance, Rue Verte 2, 1205 Geneva, Switzerland
[c]Fondation Phénix, Route de Chêne 100, 1224 Geneva, Switzerland
[d]Psychiatry Department, University of Geneva, Ch. du Petit-Bel-Air 2, 1225 Chêne-Bourg, Switzerland

Abstract

Saliva was tested and evaluated as a biological matrix for methadone (Mtd) monitoring. Conventional method using a narrow bore C_{18} column, and an enantioselective method using a narrow bore α_1-acid glycoprotein column, were developed using liquid chromatography coupled with a mass spectromeric (MS) detector. After optimisation of MS conditions by flow injection analysis, selected ion monitoring detection was used to enhance sensitivity. The total Mtd concentration and the enantiomeric ratio in saliva were validated using an experimental design. The methods were applied to samples provided by heroin addicts undergoing a Mtd treatment. Results on total Mtd determination showed a very poor correlation between saliva and serum, whereas the enantiomeric ratios of Mtd gave a very good one. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Enantiomer analysis; Methadone

1. Introduction

Methadone (Mtd) is a synthetic opiate used in drug addict maintenance programs and in the management of severe pain. In Switzerland, there are over 30 000 drug abusers of which ~15 000 are undergoing a Mtd treatment. Mtd possesses one asymmetric carbon, as shown in Fig. 1, and the analgesic potency of R-Mtd is recognised as being 50 times greater than S-Mtd [1,2]. However, in most countries, Mtd is administered as racemate. Because of important interindividual variability [3–8] and in order to prevent withdrawal symptoms, a dose adjustment is often required. Thus, it is particularly important to develop analytical methods which can determine the total Mtd concentration and the enantiomeric ratio.

Liquid chromatography (LC) methods have already been developed for conventional and stereoselective determination of Mtd [1,2,9–18]. Other techniques, such as GC or CE have also been used. Mtd was analysed in several biological fluids, such as serum [13,16,19–21], plasma [10,14,17,18,22–26], urine [19,25–28], hair [11,19], sweat [12,29] and saliva [29,30]. In comparison to

*Corresponding author. Tel.: +41-22-702-6336; fax: +41-22-781-5193.
E-mail address: Jean-Luc.Veuthey@pharm.unige.ch (J.-L. Veuthey)

0021-9673/00/$ – see front matter © 2000 Elsevier Science B.V. All rights reserved.
PII: S0021-9673(99)01001-8

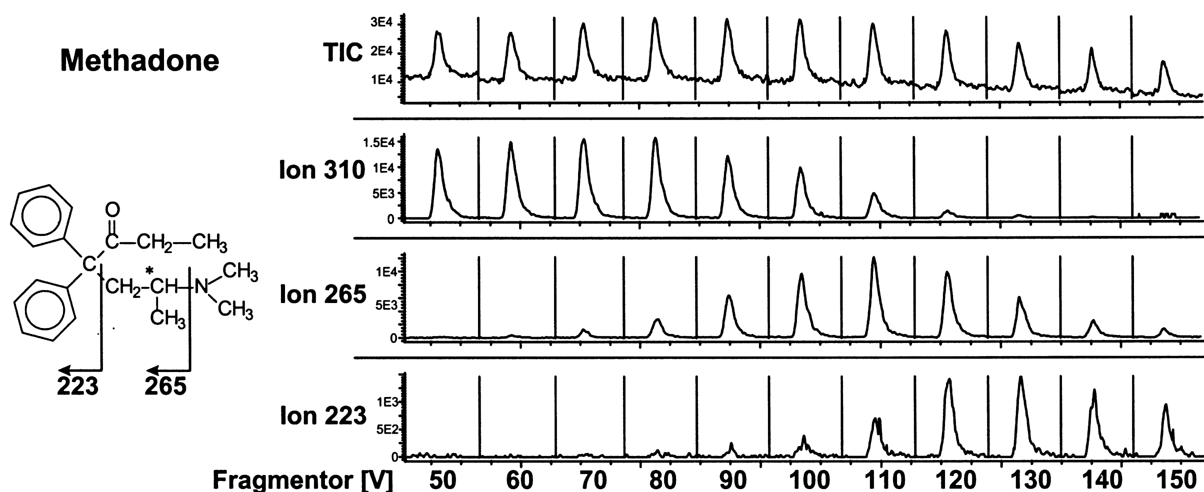

Fig. 1. Structure of Mtd (M_r 309.5) showing the position of the chiral carbon atom (asterisk) and flow injection analysis of Mtd fragmentation.

serum, saliva is a clean matrix. Saliva is constituted of 98% water and the protein concentration is of about 0.3 g/100 ml, while serum is constituted of 91% water and has a protein concentration higher than 6 g/100 ml [31]. Moreover, saliva analysis implies a non-invasive collection technique, and is therefore very attractive for drug addict patients. A number of studies have shown the applications of drug monitoring in saliva [31–37]. For some drugs (i.e. amphetamines, barbiturates, benzodiazepines), a good correlation between plasma and saliva concentrations was demonstrated. In the case of Mtd monitoring in saliva, Wolff and Hay [29] have found a correlation (r=0.8) in 21 patients.

To our knowledge, no enantioselective studies have been carried out for saliva and only the total drug concentration has been determined and compared to serum concentration.

Liquid chromatography–mass spectrometry (LC–MS) is known to be a powerful separation and detection technique in a large number of analytical fields, and particularly for the determination of drugs in biological fluids [38–42]. Its high sensitivity and selectivity present several advantages.

In this paper, a saliva evaluation was conducted for Mtd monitoring. The analyses were carried out by LC–MS using an atmospheric pressure ionisation electrospray (ESI) interface. Optimisation of the mobile phases employed, as well as of detection conditions was carried out. Saliva and serum correlations were tested for total concentration and enantiomeric ratio of Mtd.

2. Materials and methods

2.1. Chemicals

Racemic methadone hydrochloride was obtained from Hänseler (Herisau, Switzerland), R-methadone hydrochloride was a gift from Hoechst (Frankfurt am Main, Germany) and S-methadone hydrochloride was kindly supplied by Dr. C.B. Eap from the Unit of Biochemistry and Clinical Psychopharmacology (Lausanne, Switzerland). Acetonitrile and isopropanol were obtained from ROMIL (Kölliken, Switzerland). Trifluoroacetic acid (TFA) was purchased from J.T. Baker (Phillipsburg, USA). *N,N*-Dimethyloctylamine, acetic acid and ammonium hydroxide were purchased from Fluka (Buchs, Switzerland). Water was obtained using a Milli-Q gradient A10 apparatus (Millipore, Molsheim, France). Other chemicals were of HPLC or analytical grade and were used without any further purification.

2.2. Sample collection

Saliva and serum samples were obtained from

patients undergoing a methadone treatment and were provided by the Division d'abus de substance (Geneva, Switzerland) or by the Fondation Phénix (Geneva, Switzerland). Racemic methadone was administered orally either as a liquid, a tablet or a suppository. The dose range of racemic Mtd was between 15 to 305 mg per day. Blood and saliva samples were taken from each patient after three weeks of administration, so that a steady state was reached and were collected 24 h after the last intake.

Salivette (Sarstedt, Sevelen, Switzerland) was used for saliva collection. After being soaked for 2 min, the cotton roll was placed in the container and centrifuged 5 min at 1000 *g*. The volume of saliva collected was between 100 and 1500 μl. The clear saliva was frozen at −20°C. The blood was collected in a glass tube and the serum was obtained by 10 min centrifugation at 1000 *g* and stored at −20°C. Blank saliva and serum samples were obtained from an healthy volunteer.

2.3. Sample preparation

For the saliva, sample preparation was achieved by a simple filtration. 100 μl of saliva was filtered on a 0.2 μm nylon membrane filter (Millipore) and directly injected in the LC–MS system. The serum sample was extracted using a liquid–liquid extraction procedure, a technique already published [9], and was analysed by LC–UV. For saliva and serum analysis, the same sample was used for the conventional and enantioselective determinations.

2.4. Liquid chromatography

A LC system HP Series 1100 (Hewlett-Packard, Palo Alto, CA, USA) with UV detector was employed for all experiments. For MS detection, a HP Series 1100 MSD (Hewlett-Packard) equipped with electrospray source and orthogonal sprayer was used. HP Chemstation software, version 6.01, was used for instrument control, data acquisition and handling.

For the conventional analysis, a C_{18}AB Nucleosil 100, 5 μm, 125×2 mm I.D. (Macherey–Nagel, Düren, Germany) and a precolumn 10×2 mm containing the same stationary phase were used. The mobile phase was constituted of TFA 0.1% in water–MeCN (60:40, v/v), using a flow-rate of 0.25 ml/min. 2 μl of filtered saliva was directly injected in the LC–MS system.

For the enantioselective analysis of Mtd, a chiral α_1-acid glycoprotein (AGP) AGP, 5 μm, 100×2 mm I.D. column (ChromTech, Hägersten, Sweden) and a 10×2 mm precolumn containing the same stationary phase were used. For the saliva analysis, the mobile phase was constituted of a 0.01 *M* ammonium acetate buffer, containing 0.05% of *N*,*N*-dimethyloctylamine set at pH 6.6–isopropanol (85:15, v/v), and using a flow-rate of 0.25 ml/min. 5 μl was injected and MS detection was carried out.

MS conditions were the same for the conventional and enantioselective analysis of saliva. Detection of the molecular ion $M+H^+$ 310 (Mtd) was conducted. The other optimised parameters were fragmentation voltage of 70 V in positive mode, drying gas temperature at 350°C, drying gas flow-rate of 8 l/min, nebulisation pressure of 25 p.s.i. and capillary voltage of 3000 V (p.s.i. = 6894.76 Pa).

2.5. Validation procedure

Since only the enantiomeric ratio has to be determined in saliva, the latter was validated in two steps. Firstly, linearity, repeatability and reproducibility of the ratio determination was evaluated between 0.11 and 9 (respectively 10 and 90% of R-Mtd) with a constant Mtd concentration of 500 ng/ml. Secondly, the evaluation of the ratio determination robustness according to the total Mtd concentration, was conducted using an experimental design.

2.5.1. Linearity

Linearity was carried out over 3 days. The measured R/S ratio, determined as the peak area ratio, was reported as a function of the theoretical ratio. Five spiked saliva standards, prepared thrice at a constant methadone concentration of 500 ng/ml and containing various R/S ratios (0.11, 0.43, 1, 2.33 and 9), were injected. Variance homoscedasticity was verified using a Cochran test for the ratios determined each day, as well as over 3 days. In function of the results, a linear regression analysis can be applied. The values of the slope and intercept were tested using a Student *t*-test, at a confidence interval of 95%.

2.5.2. Repeatability and recovery

On each day, three quality control samples were injected after being prepared four times with a R/S ratio equal to 0.25, 1 and 4. These standards were used to calculate the recovery, the repeatability and the reproducibility of the method. Each value was recalculated using the regression line of the corresponding day. Recovery values were obtained by dividing the recalculated R/S ratio by the theoretical ratio multiplied by 100. For each concentration, the mean value was calculated over the 3 days. Repeatability and reproducibility were calculated according to the SFSTP procedure [43].

2.5.3. Experimental design

In order to test the ratio determination performance on a wider range of Mtd concentrations, an experimental design procedure was used. The concentration of Mtd varied from 50 to 950 ng/ml and the R/S ratio from 0.11 to 9. The experimental design selected was a central composite design with two factors (concentration and R/S ratio). The center point (R/S ratio = 1) was repeated five times and all the other points were carried out four times to evaluate the repeatability, and the influences of the two studied parameters, on the response. Thirty seven experiments were performed randomly and two responses studied: relative accuracy [(measured ratio/theoretical ratio)·100] and repeatability. All results were treated using Nemrod 3.0 software (LPRAI, Marseille, France). The equations obtained by multidimensional regression were expressed as a polynomial function $y = b_0 + b_1x_1 + b_2x_2 + b_{11}x_1^2 + b_{22}x_2^2 + b_{12}x_1x_2$, where x_1 corresponds to the theoretical R/S ratio, and x_2 to the total Mtd concentration. Response surfaces were drawn for each studied response.

3. Results and discussion

3.1. Optimisation of LC–ESI-MS parameters

For the determination of total Mtd, LC–ESI-MS analysis used a mobile phase containing 0.1% TFA in water–acetonitrile (60:40, v/v). For the enantioseparation of methadone enantiomers, an ammonium acetate buffer solution (10 m*M*) was employed containing 0.05% of *N,N*-dimethyloctylamine (DMOA) set at pH 6.6,–isopropanol (85;15, v/v). In order to enhance the sensitivity of the MS detection, the concentration of DMOA was optimised, the latter acting as an ionisation inhibitor. Indeed, sensitivity was five times better without DMOA than with 0.1% of DMOA. But, the use of this modifier dirties the nebulisation chamber and which therefore requires a daily maintenance to preserve the quality of the analysis. However, DMOA is necessary to ensure optimal efficiency and resolution between enantiomers. DMOA concentration was set at 0.05% and the isopropanol percentage in the mobile phase was set at 15% for a short analysis time. 5 μl was injected in the LC system. The flow-rate was set at 0.25 ml/min and the temperature at 25°C. After 200 injections, the back-pressure and the performance of the system were not modified.

The optimisation of the mass spectrometer parameters was carried out by flow injection analysis (FIA). Fragmentation voltage (50–150 V), nebulisation pressure (6–60 p.s.i.), drying gas pressure (3–13 l/min), as well as capillary voltage (2000–6000 V) were tested. These parameters are of great importance for the optimal nebulisation of the solution and ionisation of the analyte. An aqueous standard solution, containing 10 ppm of Mtd, was injected every 1.2 min. Between each injection, the studied parameter was incremented.

Fragmentation voltage is an important parameter for MS detection with ESI, since it allows one to induce molecule fragmentation and enhance the selectivity. Indeed, in addition to the molecular ion $(M+H^+)$, some fragments can be observed. But, in order to reach the best sensitivity, the fragmentation of the molecular ion is not desirable. The fragmentation voltage for Mtd was studied between 50 and 150 V with results shown in Fig. 1. Up to a fragmentation voltage of 70 V, only molecular ion 310 was observed. Above this voltage two fragments appear with masses of 265 and 223. The best sensitivity of the detector was reached with a voltage of 70 V. The nebulisation pressure was also varied from 6 to 60 p.s.i. It was observed that below 10 p.s.i., nebulisation was not regular. Therefore, the background signal increased considerably and sensitivity decreased. This pressure was set at 25 p.s.i. for all the following investigations. The drying gas temperature

was fixed at 350°C. This high temperature did not decompose Mtd which is not thermolabile. The drying gas flow-rate was varied between 3 and 13 l/min. Below 6 l/min, the evaporation of the mobile phase was not complete. Consequently, a background noise was created and the signal was reduced. A value of 8 l/min was set for all the following investigations. The capillary voltage was varied between 2000 and 6000 V. This parameter had no influence and a value of 3000 V was selected. In these conditions, sensitivity was good and only a very small amount of Mtd (1 ng/ml) was detected. Fig. 2 shows the LC–MS of blank saliva (a), saliva spiked with 100 ng/ml of racemic Mtd (b) and patient saliva (c).

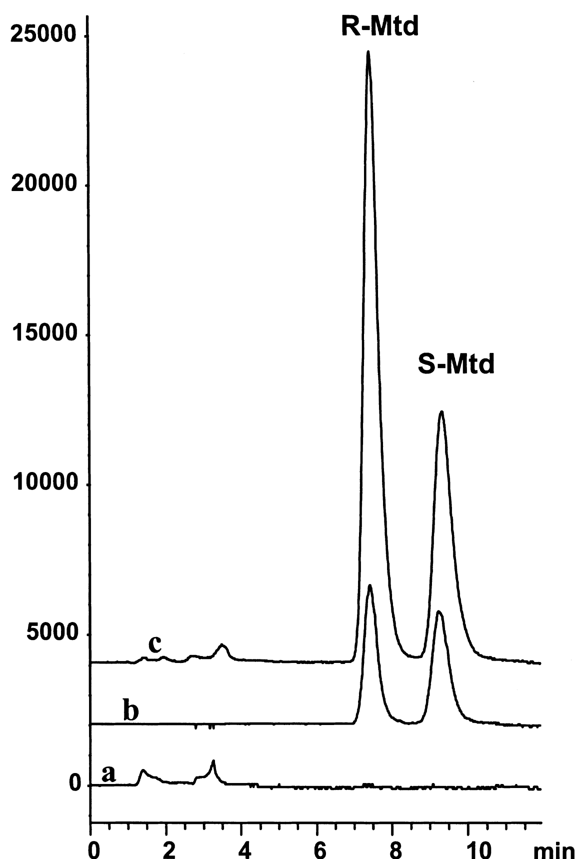

Fig. 2. (a) Blank saliva, (b) saliva spiked with 100 ng/ml of racemic Mtd, (c) patient saliva. R-mtd: t_R=7.5 min, k'=7.8, N=2100; S-mtd: t_R=9.3 min, k'=9.9, N=2080; Rs=2.45, α=1.3. LC parameters: as described in Section 2.4. MS parameters: fragmentation tension of 70 V in positive mode, drying gas temperature of 350°C, drying gas flow-rate of 8 l/min, nebulisation pressure of 25 p.s.i. and capillary voltage of 3000 V.

3.2. Conventional analysis

The total determination of Mtd in saliva was tested by LC–MS and gave good results on spiked samples. After more than 100 injections of saliva samples, chromatographic stability and reproducibility did not change significantly, nor was there any relevant change in column back-pressure. The method was applied to 28 saliva samples and the results were compared to those obtained with serum. As shown in Fig. 3, no correlation was observed between serum and saliva concentrations. These results are in agreement with the theory described in the literature [35], concerning the transport of a drug through lipid membranes which separate the saliva ducts from the systemic circulation. When equilibrium is reached, saliva/plasma ratio depends on the molecule pK_a, on the plasma and saliva pH, as well as on the free drug concentration (Eq. (1)):

$$\frac{\mathrm{Drug}_{\mathrm{saliva}}}{\mathrm{Drug}_{\mathrm{plasma}}} = \frac{1+10^{(\mathrm{p}K_a - \mathrm{pH}_{\mathrm{saliva}})}}{1+10^{(\mathrm{p}K_a - \mathrm{pH}_{\mathrm{plasma}})}} \cdot \frac{\mathrm{Free.Drug}_{\mathrm{plasma}}}{\mathrm{Free.Drug}_{\mathrm{saliva}}} \quad (1)$$

Therefore, saliva is only representative of the free fraction of drug in plasma. However, saliva pH (ca. 6.5), as well as its volume, can vary dramatically according to the sample collection (stimulation, chewing gum, etc.) and to previous ingestion. Furthermore, saliva monitoring is not appropriate for compounds of pK_a which are close to the saliva and blood pH. In the case of Mtd (pK_a 8.3), a weak variation of the saliva pH can involve a modification of the Mtd passive diffusion in saliva. This is why a poor correlation was observed between total drug concentrations.

3.3. Enantioselective analysis

Despite the fact that no correlation was found in the total Mtd concentration between saliva and serum, an enantioselective determination was carried out to evaluate the R/S ratio correlation for both matrices. The ratio determination was validated and

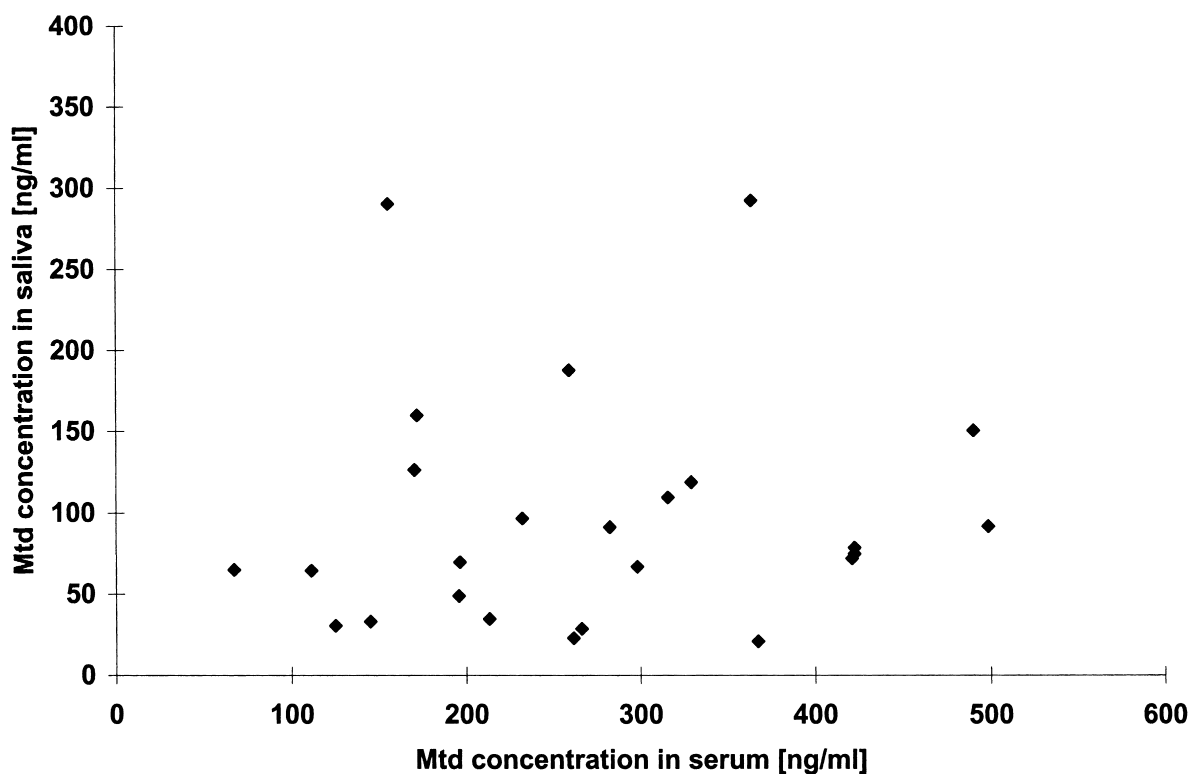

Fig. 3. Correlation between saliva and serum Mtd concentrations for 28 patients.

the procedure applied to patients participating in a clinical study.

3.3.1. Linearity

The variances of the measured ratios were homogeneous in the tested range (ratio from 0.11 to 9) for the 3 days. The linear regression was calculated and gave the following equations:

Day 1: $y = 0.9776x + 1.4407$ ($R^2 = 0.9997$)

Day 2: $y = 0.9789x + 1.0718$ ($R^2 = 0.9998$)

Day 3: $y = 0.9909x + 0.5453$ ($R^2 = 0.9991$)

Recalculated values exhibited a RSD inferior to 3% at each concentration level. The theoretical R/S ratio was then reported in function of the recalculated R/S ratio. A Student *t*-test was applied: the slope was not different from 1 and the intercept not significantly different from zero. The linear model proved thus to be completely suitable.

3.3.2. Repeatability and recovery and limit of quantification

The results obtained are summarised in Table 1. The recovery values included all the 100% at a confidence interval (C.I.) of 95%. The highest values for repeatability and reproducibility were obtained for the ratio 0.25 and were equal to 3.0% and 5.1%, respectively. The ratio determination is repeatable and reproducible at a constant Mtd concentration of 500 ng/ml. Using MS detection in the SIM mode, the absolute limit of quantification of methadone, expressed as a signal-to-noise ratio of 10, was 5 ng/ml. The use of MS detection allowed a gain of about 50 in absolute determination of methadone compared to UV detection [21].

Table 1
Recovery, repeatability and reproducibility values $k=3$, $n=4$

R/S ratio	Within-day			Between-day			
	Day 1	Day 2	Day 3	Mean±C.I.	Recovery±C.I.% (%)	Repeatability (%)	Reproducibility (%)
0.25	0.27	0.25	0.24	0.25±0.01	100.4±2.91	3.0	5.1
1.00	1	1.02	1	1.00±0.02	100.2±0.82	1.4	1.4
4.00	3.95	3.95	3.99	3.96±0.13	99.8±0.63	1.1	1.1
				Mean	100.1±1.76	1.8	2.5

3.3.3. Robustness

An experimental design was used to test the robustness of the ratio determination, according to the concentration of total Mtd in the sample. Response surfaces can be drawn, as shown in Figs. 4 and 5. The relative accuracy was between 95 and 105% in the studied domain, except for samples with low concentration (<200 ng/ml) and low R/S ratio (<0.4) and the mean response was 102.3%. The response surface for repeatability was rather similar. Again, the highest error was observed for low concentration and low R/S ratio samples. The mean repeatability was 1.0%. Considering these results, the ratio determination was very slightly influenced by the sample concentration. Therefore, the ratio determination is robust, repeatable and reproducible in the therapeutic domain of Mtd in saliva.

3.3.4. Application to clinical cases

Determination of R/S ratio in saliva was carried out on 100 clinical samples. The results obtained were compared with those determined in serum by an enantioselective LC–UV procedure published previously [9]. Fig. 6 shows a good correlation ($r=0.91$) between these ratios. However, the ratio observed in saliva was included between 1 and 9, while in serum this ratio was between 0.5 and 3. This

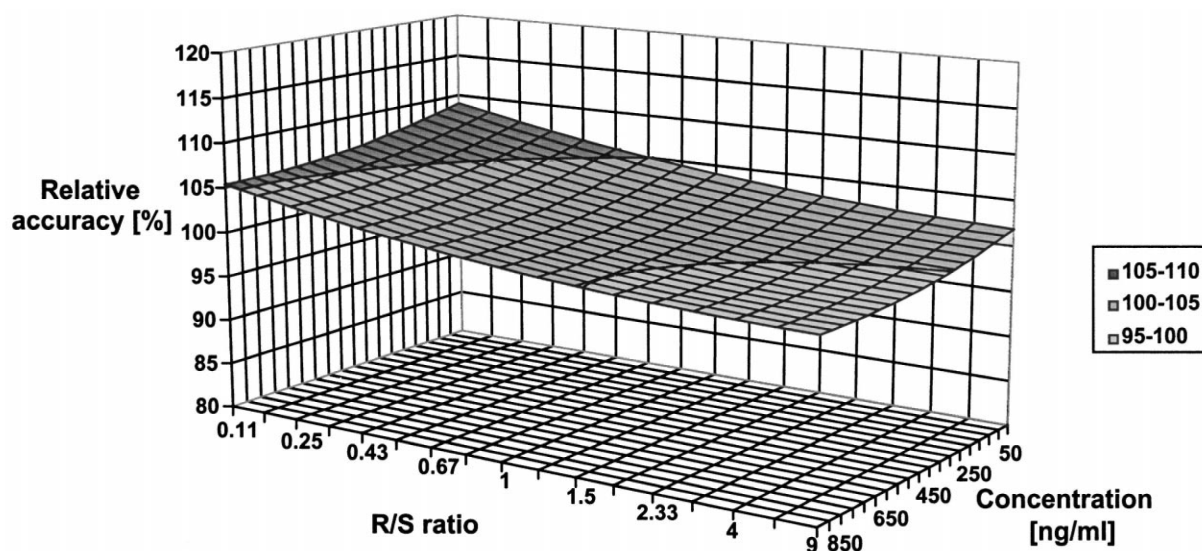

Fig. 4. Recovery response surface.

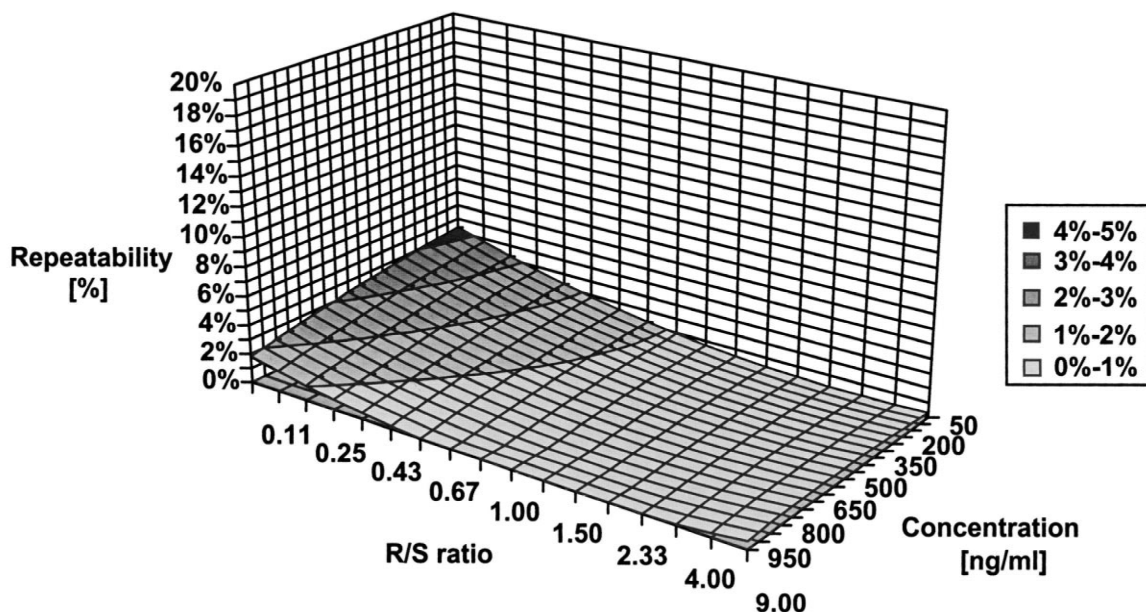

Fig. 5. Repeatability response surface.

result can be easily explained since it has been reported that the ratio determined in saliva is representative of the free methadone fraction only. Because in blood S-Mtd is preferentially bounded to proteins [44], its free fraction is reduced and results in a higher R/S ratio in saliva.

Measuring the R/S ratio of Mtd in saliva with this procedure presents many advantages. The method is very fast and costless, because of the absence of sample preparation, and results in a high throughput sample procedure. Moreover, as a non-invasive technique, the method is very attractive for patients. In the case of Mtd, the serum R/S ratio, which interests the clinician, can be easily obtained by measuring the saliva R/S ratio as shown in Fig. 6. This fast R/S ratio determination can be complementary to a total determination of Mtd concentration by radio immunoassay for example, as it also does not require a sample preparation.

4. Conclusion

An achiral and an enantioselective method were developed for the determination of Mtd in saliva. Coupling mass spectrometry to liquid chromatography allowed detection of very low concentrations of compounds. Thus, there was no need for a sample preconcentration step before analysis. A sample filtration was sufficient to avoid clogging of the chromatographic column or of the MS interface capillary. The detector selectivity enabled to simplify the extraction procedure. An optimisation of mobile phase and MS conditions was conducted by FIA. The enantioselective analysis was validated and showed very good results. The method was applied to samples taken from heroin addicts undergoing a Mtd treatment. Results exhibited a very poor correlation between saliva and serum total Mtd concentration. The enantiomeric ratio of Mtd in saliva was established and showed a good correlation with the R/S ratio of serum. Using saliva makes it easy to determine the enantiomeric ratio of Mtd. The R/S ratio measured in saliva is always higher because this matrix reflects the unbound fraction in serum. Saliva samples are more convenient as they allow a non-invasive collection, and require a rapid, low cost and easy sample preparation. Furthermore, using LC–MS induces very good repeatability and reproducibility.

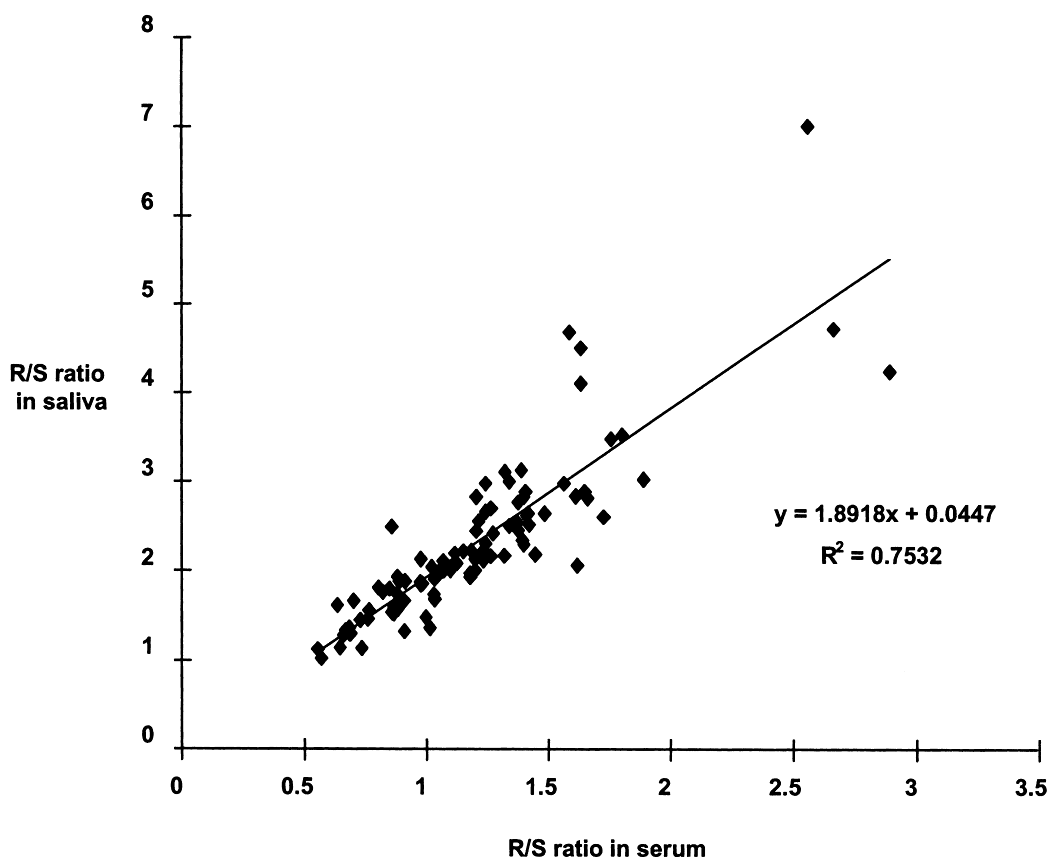

Fig. 6. Correlation between saliva and serum ratios.

References

[1] H.F. Fraser, H. Isbell, Bull. Narc. 14 (1962) 25.
[2] N.B. Eddy, H. Halbach, O.J. Braeden, Bull. WHO 14 (1957) 353.
[3] C.B. Eap, T. Finkbeiner, M. Gastpar, N. Scherbaum, K. Powell, P. Baumann, Eur. J. Clin. Pharmacol. 50 (1996) 385.
[4] C.B. Eap, G. Bertschy, P. Baumann, T. Finkbeiner, M. Gastpar, N. Scherbaum, Arch. Gen. Psychiatry 55 (1998) 89.
[5] J.W. de Vos, P.J. Geerlings, W. van der Brink, J.G. Ufkes, H. van Wilgenburg, Eur. J. Clin. Pharmacol. 48 (1995) 361.
[6] K. Wolff, A. Rostami-Hodjegan, S. Shires et al., Br. J. Clin. Pharm. 44 (1997) 325.
[7] K. Kristensen, T. Blemmer, H.R. Angelo et al., Ther. Drug Monit. 18 (1996) 221.
[8] K. Wolff, A.W. Hay, D. Raistrick, R. Calvert, Eur. J. Clin. Pharmacol. 44 (1993) 189.
[9] S. Rudaz, D. Ortelli, M. Gex-Fabry, J.-J. Déglon, L.P. Balant, J.-L. Veuthey, Chirality 11 (1999) 487.
[10] O. Beck, L.O. Boreus, P. Lafolie, G. Jacobsson, J. Chromatogr. 570 (1991) 198.
[11] P. Kintz, H.P. Eser, A. Tracqui, M. Moeller, V. Cirimele, P. Mangin, J. Forensic Sci. 42 (1997) 291.
[12] P. Kintz, A. Tracqui, C. Marzullo, A. Darreye, F. Tremeau, P. Greth, B. Ludes, Ther. Drug Monit. 20 (1998) 35.
[13] K. Kristensen, H.R. Angelo, T. Blemmer, J. Chromatogr. A 666 (1994) 283.
[14] R.L.G. Norris, P.J. Ravenscroft, S.M. Pond, J. Chromatogr. B 661 (1994) 346.
[15] C. Pham-Huy, N. Chikhi-Chorfi, H. Galons et al., J. Chromatogr. B 700 (1997) 155.
[16] S. Rudaz, J.-L. Veuthey, J. Pharm. Biomed. Anal. 14 (1996) 1271.
[17] N. Schmidt, K. Brune, G. Geisslinger, J. Chromatogr. 583 (1992) 195.
[18] T.L. Pierce, A.G.W. Murray, W. Hope, J. Chromatogr. Sci. 30 (1992) 443.
[19] M. Frost, H. Kohler, G. Blaschke, Electrophoresis 18 (1997) 1026.
[20] K. Kristensen, H.R. Angelo, Chirality 4 (1992) 263.
[21] S. Rudaz, J.-L. Veuthey, Chromatographia 44 (1997) 283.
[22] O. Beck, L.O. Boreus, S. Borg, G. Jacobsson, P. Lafolie, M. Stensio, Ther. Drug Monit. 12 (1990) 473.

[23] C.E. Inturrisi, K. Verebely, J. Chromatogr. 65 (1972) 361.
[24] P. Kintz, A. Tracqui, A.J. Lugnier, P. Mangin, A.A. Chaumont, Meth. Find. Exp. Clin. Pharmacol. 12 (1990) 193.
[25] T.L. Pierce, W. Hope, Pharmacol. Biochem. Behav. 49 (1994) 1101.
[26] N. Schmidt, R. Sittl, K. Brune, G. Geisslinger, Pharm. Res. 10 (1993) 441.
[27] S. Molteni, J. Caslavska, D. Allemann, W. Thormann, D. Alleman, J. Chromatogr. B 658 (1994) 355.
[28] W. Thormann, M. Lanz, J. Caslavska, P. Siegenthaler, R. Portmann, Electrophoresis 19 (1998) 57.
[29] K. Wolff, A.W.M. Hay, Clin. Chem. 37 (1991) 1297.
[30] G.I. Kang, F.S. Abbott, J. Chromatogr. 231 (1982) 311.
[31] K.M. Höld, D. de Boer, J. Zuidema, R.A.A. Maes, Int. J. Drug testing 1 (1996) 1.
[32] E.J. Cone, Ann. NY Acad. Sci. 694 (1993) 91.
[33] M. Danhof, D.D. Breimer, Clin. Pharmacokinet. 3 (1978) 39.
[34] N. El-Guebaly, W.J. Davidson, H.A. Sures, W. Griffin, Can. J. Psychiatry 26 (1981) 43.
[35] D.A. Kidwell, J.C. Holland, S. Athanaselis, J. Chromatogr. B 713 (1998) 111.
[36] J.C. Mucklow, M.R. Bending, G.C. Kahn, C.T. Dollery, Clin. Pharmacol. Ther. 24 (1978) 563.
[37] W. Schramm, R.H. Smith, P.A. Craig, D.A. Kidwell, J. Anal. Tox. 16 (1992) 1.
[38] H. Hoja, P. Marquet, B. Verneuil, H. Lotfi, B. Penicaut, G. Lachatre, J. Anal. Tox. 21 (1997) 116.
[39] M.S. Lee, E.H. Kerns, M.E. Hail, J. Liu, K.J. Volk, LC·GC 15 (1997) 542.
[40] H.H. Maurer, J. Chromatogr. B 713 (1998) 3.
[41] M. Nishikawa, H. Tsuchihashi, J. Toxicol. 17 (1998) 13.
[42] S. Zhou, M. Hamburger, J. Chromatogr. A 755 (1996) 189.
[43] E. Chapuzet, N. Mercier, S. Bevoas-Martin et al., S.T.P. Pharma Pratiques 7 (1997) 169.
[44] M.K. Romach, K.M. Piafsky, J.G. Abel, V. Khouw, E.M. Sellers, Clin. Pharmaco. Ther. 29 (1981) 211.